SURVIVAL AND GROWTH OF LARVAE OF THE EUROPEAN OYSTER, O. EDULIS, AT LOWERED SALINITIES

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The European oyster, *Ostrea edulis*, in its native habitat, is found primarily in oceanic or nearly oceanic salinities. Its native range extends from the southern coast of England and the Scandinavian countries to the Mediterranean, but recently it has been successfully introduced into New England waters (Loosanoff, 1951, 1952, 1955; Welch, in press).

Initially, Loosanoff (1955) proposed this oyster for introduction into those areas where the summer water temperatures rarely, if ever, are high enough for American oysters to reproduce. More recently, because of heavy mortalities of American oysters in several states, he has suggested that *O. edulis* might be introduced as a second commercial oyster into these and other oyster-producing areas of the United States. Since European and American oysters belong to different genera, they will not interbreed and, what could be extremely important, European oysters might not be susceptible to some of the diseases now affecting American oysters. Moreover, since European oysters are larviparous and their larvae are usually 175 μ to 185 μ at the time of release, the food requirements of the larvae are not as restricted as those of the young larvae of American oysters. Therefore, good sets of European oysters might frequently be obtained in seasons when setting of American oysters fails.

Korringa (1941) reviewed the literature on the effects of salinity on eggs and larvae of several species of oysters, including *O. edulis*. He found no correlation between rate of growth of larvae or intensity of their setting and differences in average salinities in the Oosterschelde, Holland, ranging from 25 to 35 ppt. He quotes the assumption of Gaarder (1932, 1933) and Gaarder and Bjerkan (1934) that 24 ppt is the lowest salinity for satisfactory growth of larvae of *O. edulis*. Korringa also states that changes of salinity, within the range found in the Oosterschelde, cannot be held responsible for the success or failure of spatfall of *O. edulis* in this area, and points out that experiments *in vitro* had not yet been carried out to determine the effect of salinity upon setting.

Walne (1956) reported experiments on rearing *O. edulis* larvae in salinities initially adjusted to 21.1, 25.9, 27.9 and 31.3 ppt. The water in the larval cultures was not changed during the course of his experiments and, apparently due to evaporation and the addition of algal food suspension, the salinity in all cultures increased. Thus, in cultures initially at 21.1 ppt the salinity increased to 25.9 and 26.2 ppt. Under these conditions he found that the larvae survived and grew

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throughout the salinity range tested, but he did not obtain any spatfall in the cultures started at 21.1 ppt.

The present study was undertaken to obtain more precise data on the effect of low salinities on egg production and incubation in *O. edulis,* upon survival and growth of larvae, and upon setting of mature larvae. Since several of the oyster-producing areas in the United States, where we might wish to introduce *O. edulis,* are characterized by relatively low salinities, such information is necessary.

Methods

The methods employed were essentially the same as those used by Davis (1958) in a similar study of the salinity tolerance of eggs and larvae of the American oyster. However, since *O. edulis* is larviparous, to obtain larvae from these oysters it was necessary to hold adults in aquaria during the period of gonad development, spawning and incubation. Approximately ten adult oysters were kept in each aquarium in 60 liters of water. One group of oysters was kept at our normal salinity (27 ppt); another group was kept at a salinity of 20 ppt; and a third group was kept at 17.5 ppt. We used sea water, filtered through an Orlon filter designed to remove particles above 15 μ in diameter, and lowered the salinity to the desired level with demineralized tap water. Water in these aquaria was changed daily and three to four liters of algae were added during each change to supplement the food present in the water.

The adult oysters kept at normal salinity provided the larvae used in these experiments. As soon as larvae were noticed after release, they were collected by draining the water from the aquarium through a 250-mesh screen. By using care to drain without disturbing the sediment on the bottom of the aquarium, healthy larvae were collected relatively free of debris. These larvae were then resuspended in a three-liter Pyrex jar, the number of larvae per ml. determined, an appropriate volume pipetted into each of a series of one-liter polyethylene beakers, and the volume made up to one liter of the desired salinity. Two beakers of larvae were set up at each of the following salinities: 27 ppt (control), 25 ppt, 22.5 ppt, 20 ppt, 17.5 ppt, 15 ppt, 12.5 ppt and 10 ppt.

In the first experiment we used 6400 and in the second, approximately 13,000 larvae per beaker. Following the procedure of Davis (1958), to hold salinities constant, the one-liter cultures received food only every second day when the water was changed, instead of daily as is the usual practice. All the cultures were covered to prevent excessive evaporation and kept in a constant temperature bath at 23° C. ± 1° C.

In the two experiments to determine the effect of low salinities on setting of mature larvae, we reared the larvae in 15-liter culture vessels at 27 ppt until their average size was 250 μ and many were already in the 275 to 300 μ range. Approximately 9000 of these larvae were pipetted into each of a series of one-liter beakers filled with water adjusted to the desired salinities. In the first experiment, because of the limited number of mature larvae available, we used a single culture at each salinity, but in the second, mature larvae were abundant and duplicate cultures were used at each salinity.

A single oyster shell in each beaker was used as cultch. These shells were replaced every second day, as the water was changed, and the shells removed

were examined under a dissecting microscope to determine the number of spat caught. Only those setting on the smooth, white, inner surface of the shell were counted. Since many oysters also set on the rough, dark, outer surface of the shell, on the walls of the container, and on small shell fragments or other debris where it is impossible to count, the number of spat recorded is only a rough index of the total number setting.

Results

Effects of lowered salinities on growth of larvae

The results of the first experiment showed that growth of larvae was virtually normal in salinities as low as 22.5 ppt (Fig. 1). At 20 ppt, although larvae grew

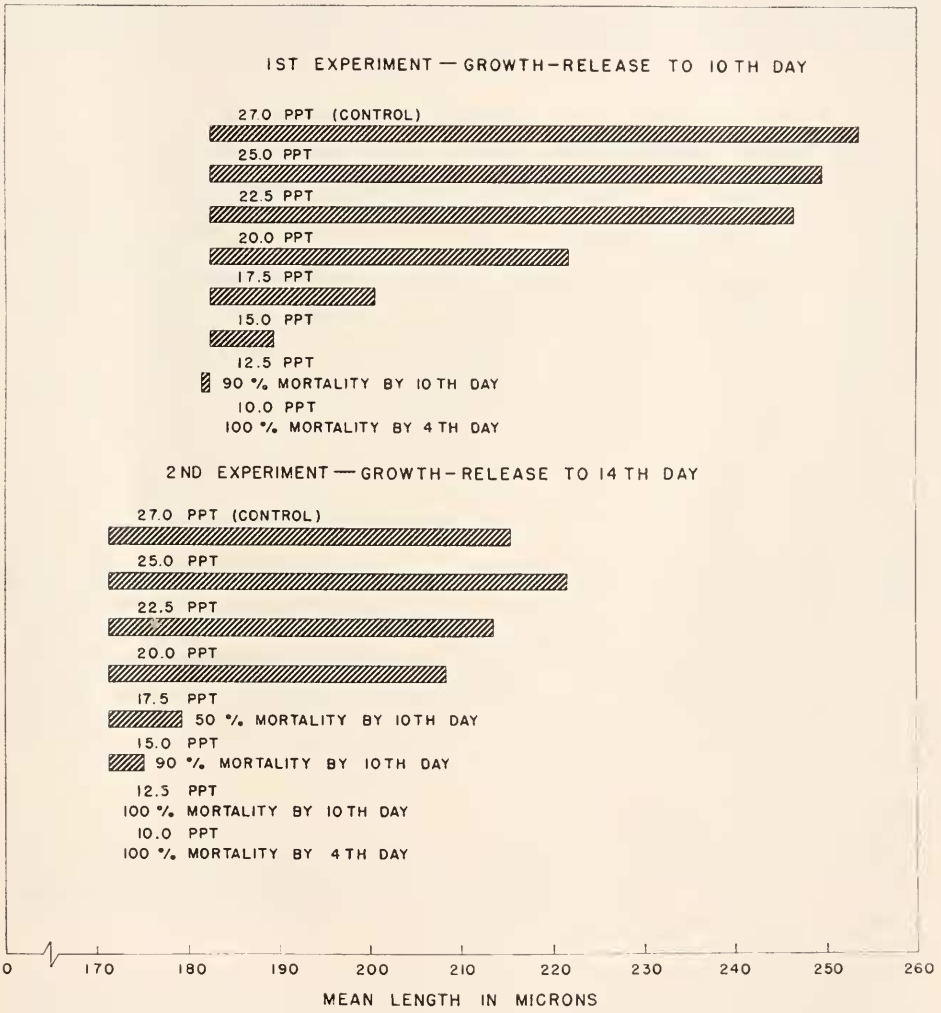

FIGURE 1. Growth of *O. edulis* larvae at different salinities. Mean lengths are based on measurements of 100 larvae from each of duplicate cultures at each salinity.

at a considerably slower rate, some were reared to setting stage and metamorphosed. At 17.5 ppt growth was extremely slow; no larvae reached the setting stage and eventually all died. At 15 and 12.5 ppt, growth of larvae was even slower, but some lived at each salinity for ten days or more. Larvae kept at 10 ppt all died within four days.

The larvae used in the second experiment were about 11 μ smaller at the time of release and grew at a considerably slower rate than those used in the first experiment (Fig. 1). Since larvae from the brood used in this second experiment grew normally in other cultures set up at the same time, we believe the slower growth was the result of the higher concentration of larvae (13,000 per liter as opposed to 6000 per liter in the first experiment). This would indicate that to assure good growth these larvae must be kept in much lower concentrations than larvae of either *Venus mercenaria* or *Crassostrea virginica,* both of which grow quite well at 13,000 individuals per liter.

TABLE I

Effect of reduced salinities on setting of larvae reared almost to setting stage at 26–27 ppt

Salinity in parts per thousand	Average number of spat per culture	
	1st experiment	2nd experiment
26–27 (Control)	147	134
25.0	504	145
22.5	120	171
20.0	114	38
17.5	24	5
15.0	4	0.5
12.5	0	0
10.0	0	0
7.5	0	0

The pattern of growth was generally the same in both experiments, even though growth of larvae was much slower in the second experiment. Growth of larvae was not greatly different from that in control cultures at salinities of 20 ppt and higher. As in the first experiment, the rate of growth dropped off sharply between 20 ppt and 17.5 ppt. Mortality was high and growth negligible at all lower salinities. Because of the very slow growth of larvae, the second experiment was discontinued before larvae in any of the cultures reached setting stage.

Effects of lowered salinities on setting

Two experiments were run to determine the minimum salinity at which larvae of *O. edulis* could set. In both, larvae that had been reared almost to metamorphosis at our normal salinity of 26–27 ppt were transferred directly to lowered salinities and their setting recorded (Table I).

Although a salinity of 20 ppt was the lowest at which larvae could be reared from release through metamorphosis, some mature larvae transferred to salinities of 17.5 and 15 ppt did set (Table I).

It is significant that all of the set obtained at 17.5 and 15 ppt occurred within four days after the larvae were transferred to these salinities, while setting continued for as long as 14 days in higher salinities. This indicates that only those larvae that were almost ready to set at the time of transfer were able to complete growth and metamorphose at these lower salinities, while all less developed larvae died. However, even those larvae that were ready to set at the time of transfer were unable to complete the process of metamorphosis at salinities of 12.5 ppt or lower.

A similar record of the number of set obtained from larvae reared at lowered salinities showed that while there was no significant mortality within ten days in a salinity of 15 ppt nor in any of the higher salinities, none of the larvae grown at 15 and 17.5 ppt survived to set. Even in cultures kept at 20 and 22.5 ppt, significantly fewer larvae succeeded in completing metamorphosis than in cultures reared at 25 ppt or in control cultures.

Effects of lower salinities on gonad development, spawning and incubation

Because it was found (Davis, 1958) that eggs from *C. virginica* that had developed gonads at salinities of about 8.75 would develop into straight hinge larvae at considerably lower salinities than would eggs from parents that had developed gonads at 26–27 ppt, we thought it worth while to attempt to induce gonad development, spawning and incubation of *O. edulis* at lowered salinities. We hoped to use larvae from oysters kept at salinities of 20 ppt and 17.5 ppt in salinity experiments parallel with those on larvae from oysters kept at our normal salinity, to determine whether the salinity tolerances of the larvae were altered by the salinity in which the parent oysters had developed gonads and spawned.

The adult *O. edulis* that were kept in aquaria at 20 ppt and at 17.5 ppt failed to release any normal living larvae. In other respects, however, transfer to these lowered salinities appeared to affect them for only a short time. Oysters transferred to 17.5 ppt, for example, failed to feed normally for only a few days, but thereafter cleared the water of the algae added as food, as did those kept at higher salinities. These oysters were kept in these salinities for 60 days and appeared normal in every respect, except that they produced no normal larvae.

Evidence of spawning of at least one female, in the group kept at 20 ppt, was observed, but no living larvae were recovered. A few empty larval shells were found on several occasions, but there were not enough of these to account for a normal brood from a single female. Although spawning was not observed in the group kept at 17.5 ppt, on several occasions highly abnormal living larvae were recovered. These larvae either had no shells at all or had small, highly abnormal ones. Nevertheless, many of them were capable of taking food and lived for several days after transfer to normal sea water. Because there were only a few of them, their further development could not be followed.

Discussion

The results of our experiments indicate that culturing of *O. edulis*, in areas where the salinity is 20 ppt or lower, cannot be successful because these salinities are too low for reproduction of this species. It is possible, nevertheless, that in

situations where there is a gradation of salinities, mature larvae, developing and growing to setting size at salinities of about 22.5 ppt or higher, could be carried by currents to salinities as low as 15 ppt and set and grow there.

Since some of the larvae reared at 20 ppt did metamorphose, it is possible that culture of *O. edulis* at this salinity might be successful, but growth of larvae would be slow and the intensity of setting reduced. Moreover, adult oysters kept at this salinity failed to give any living larvae. While it is possible that we might have had different results if we had used more oysters, the number of oysters (only 10) kept at this salinity was the same as the number kept at our normal salinity that released the several million larvae used in these experiments.

The release of abnormal veligers by oysters kept at 17.5 ppt is, we believe, positive evidence that this salinity is too low for normal larval development. It is possible that oysters acclimated to this salinity over a longer period of time might have given viable larvae. However, the results of Davis (1958) showed that keeping adult American oysters, which had developed gonads at 8.75 ppt, for only a few days at salinities of 7.5, 10 and 15 ppt altered the salinity tolerance of their eggs.

Previous investigators using, for the most part, field data have shown that *O. edulis* larvae can grow and set at salinities as high as 34 to 39.5 ppt (Mazzarelli, 1924). Korringa (1941) also states that, "It is my opinion that variations in salinity between 25 ppt and 35 ppt probably have little or no influence on larval growth and development in *Ostrea edulis*" (pp. 133–134).

Our results cannot be compared directly with those of Walne (1956) because he did not change the water and his salinities were not held constant. Using our methods, however, larvae were reared to metamorphosis and some set was obtained at salinities of 20 and 22.5 ppt, both lower than the lowest initial salinity (25.9) from which Walne obtained spatfall.

Our results confirm Korringa's opinion that a salinity of 25 ppt has no appreciable adverse effect on growth of larvae. They further show that the lower limit for good growth and setting is about 22.5 ppt, although larvae can grow to metamorphosis at 20 ppt and mature larvae can set at even lower salinities.

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Summary

1. At salinities of 25 and 22.5 ppt growth of larvae of *O. edulis* and intensity of setting was not significantly different from that in control cultures at our normal salinity of 26–27 ppt.

2. At 20 ppt growth of larvae was appreciably slower than at higher salinities and the intensity of setting was reduced.

3. At 17.5 and 15 ppt larvae lived for some time and showed appreciable growth, but they all died prior to metamorphosis.

4. At 12.5 ppt larvae showed no growth and by ten days after swarming they had suffered 90% or higher mortality.

5. At 10 ppt all larvae died in less than four days.

6. Some larvae that had been reared to setting size at a salinity of 26–27 ppt were capable of setting in salinities as low as 15 ppt.

7. No normal larvae were obtained from adult oysters kept at salinities of 20 or 17.5 ppt.

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